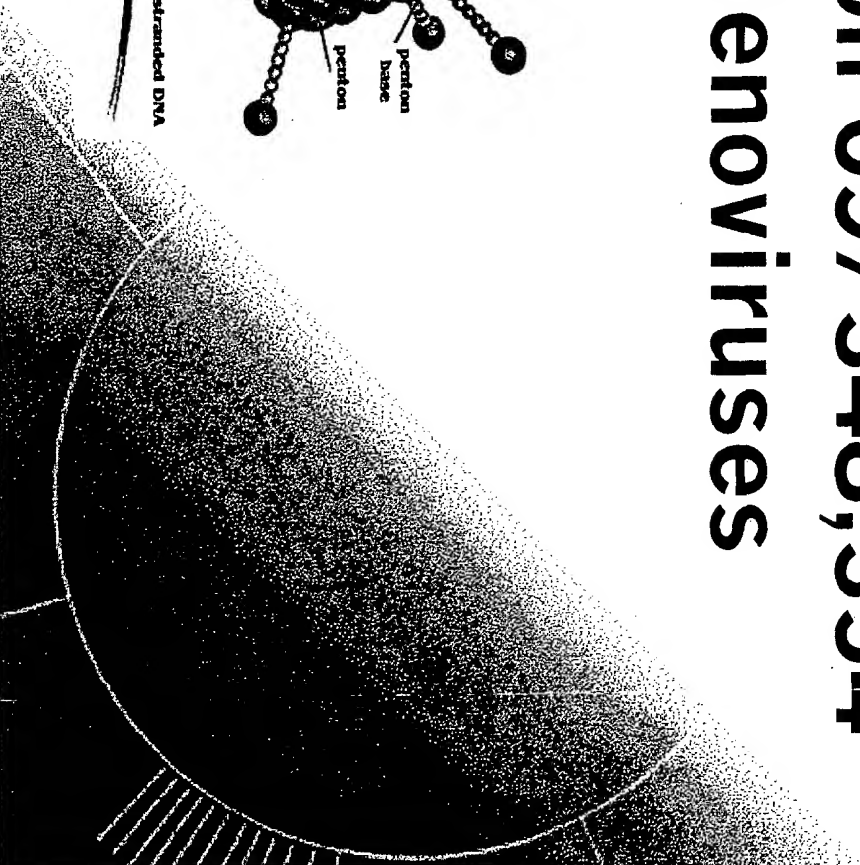
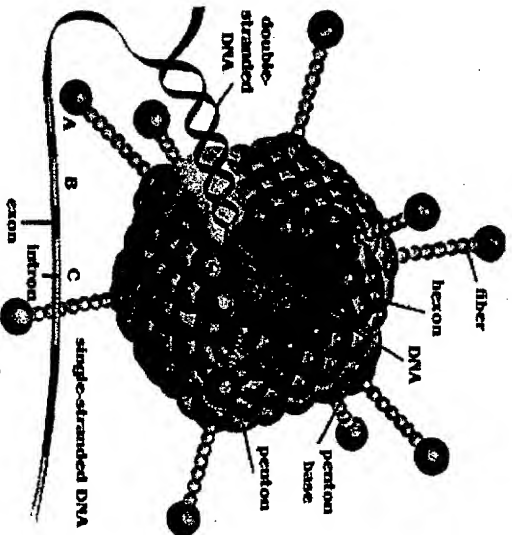


• Crucell



Patent application 09/348,354 “Chimeric adenoviruses

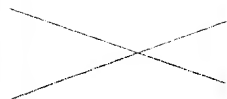


Adenovirus modified vector library

Adenovirus type 5

Transgene

FIBER



Library of Fibers

PCR amplify



7	15	33	45
8	17	34	47
9	24	35	49
10	27	37	50
11	28	38	
12	30	40-5	
13	32	40-1	

Production on PER.C6

Ad serotypes
1-51

Cruce

Definition of "Tropism"

US patent (US6,127,525):

"...receptor specificity or natural affinity for certain tissue or host organism".

Definition is limited to binding of virus to cell type due to compatibility of virus and cellular receptor.

Crucell:

The sum of biological processes that determine whether a virus can transfer a gene in vivo to a particular cell, organ or tissue.

- # Anatomical barriers
- # Viral lysis by serum components (non-antibody related)
- # Neutralizing antibodies
- # Receptor-virus compatibility
- # Vector stability

 **Crucell**

Example 1: Anatomical barriers

Due to for instance vessel wall barrier the virus is unable to reach the target of interest.

Due to the size of Adenovirus, penetration in tumor tissue is severely limited.

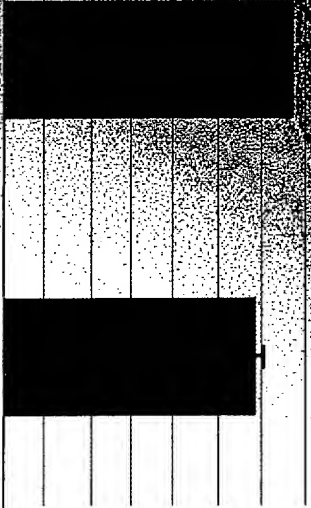
3) Expression profile of Ad5 receptor does not correlate with observed Ad5 infection patterns in rodents

(Fechner et al/ Gene Ther. 1999 Sep;6(9):1520-35)

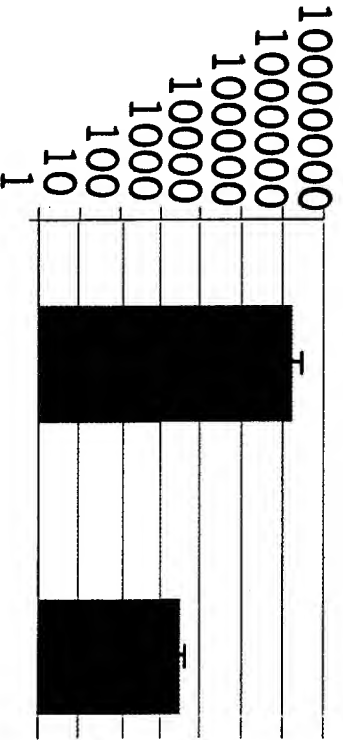
Example II: viral lysis by serum

Ad5 (pre-treatment)

Ad5



Luciferase act.



Ad5.Fib16

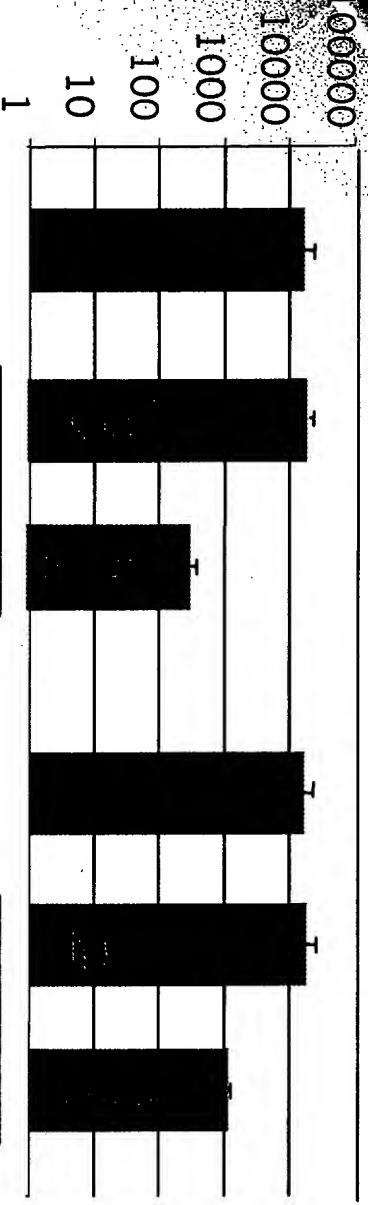
Serum +

Serum +

1/4 diluted serum, dose: 500 vp/ cell, A549 cells

Serum effect is independent of antibodies

Luciferase activity



Cynomolgus

Rhesus

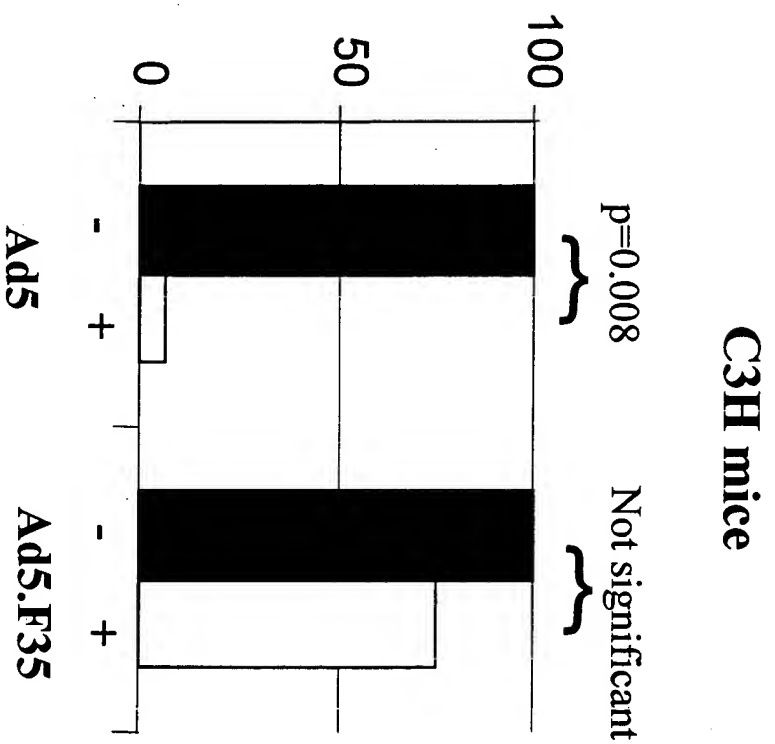
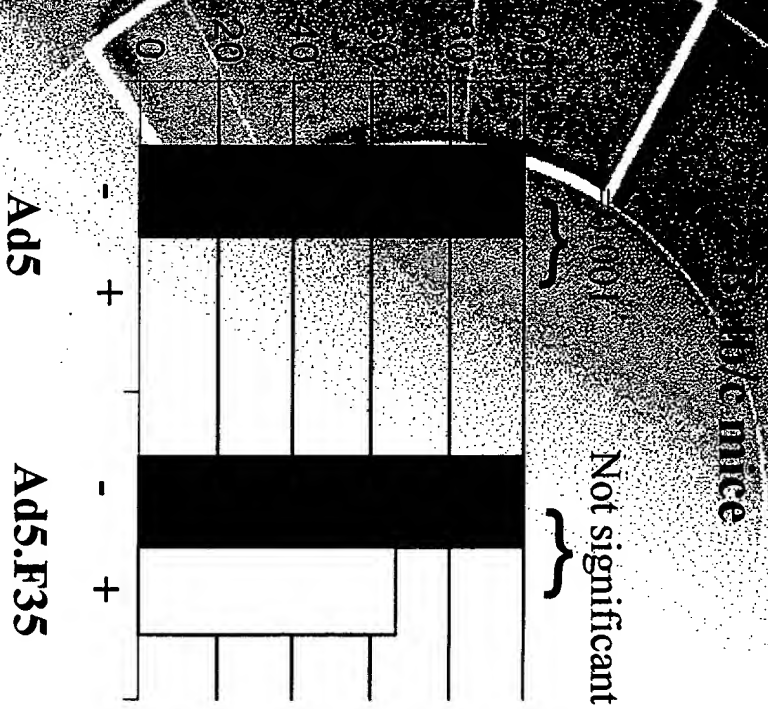
Crucell

Systemic patent (6,127,525): Concerning fiber swap and in vivo escape of Nab

Example 3 (columns 25)

These results confirm that switching the fiber from that of adenoviral serotype 5 to that of serotype 7 subgroup B vector to that of an adenoviral serotype 7 subgroup B vector by itself is sufficient to allow the vector to escape neutralising antibodies generated against an adenoviral vector comprising Ad5 fiber.....”

Ad5.F35: Neutralizing antibodies

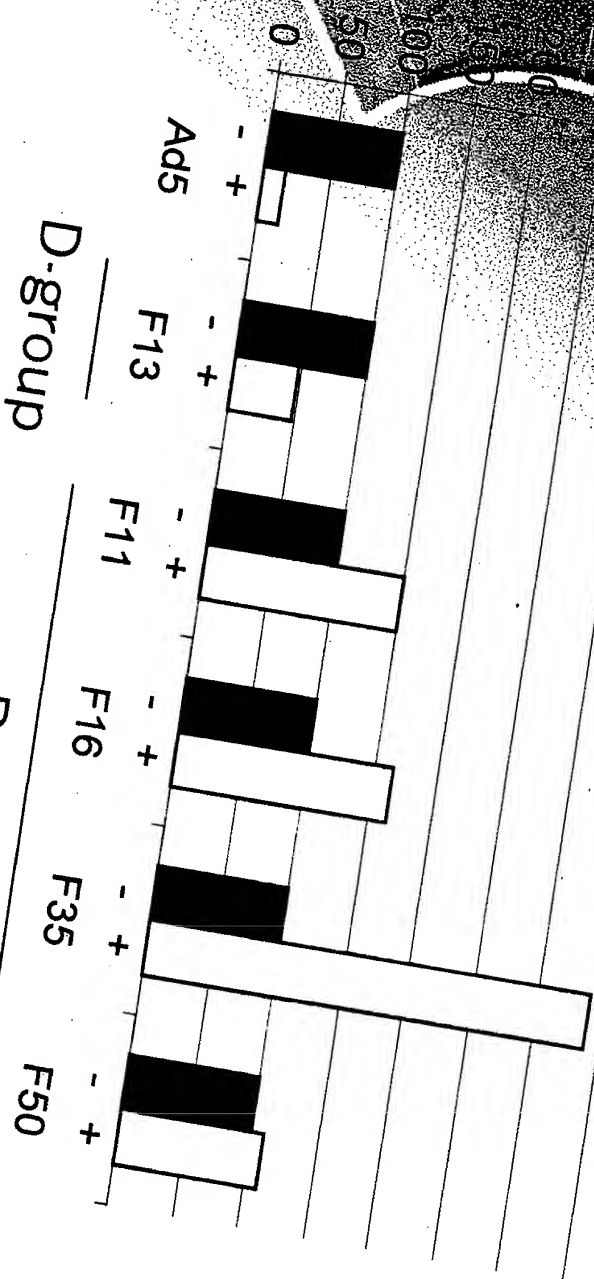


P values determined via "Mann-Whitney U" test

Effect: Neutralizing antibodies

C57/Bl6 mice

P values determined via "Mann Whitney U test"



Differences in structural design of Fiber-chimeric vector

Ad5 fiber

Complete deletion of Ad5 fiber and insertion of complete Ad7 fiber.

Ball et al.; J Virol Vol 70, p2120:

have shown that amino acid homology between the tail regions of Ad5

Ad7 is sufficient to allow functional replacement of the Ad5 fiber with Ad7

fiber

Crucell:

~ Retained Fiber tail of Ad5 to ensure proper interaction with Ad5 penton-base (i.e. homology between Ad7 and Ad5 in fiber tail region is 57% on a.a. level)

Substantial difference in vector stability expected